# Molecular Identification of Livestock Breeds: A Tool for Modern Conservation Biology

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ABSTRACT

Global livestock genetic diversity includes all of the species, breeds and strains of domestic animals, and their variations. Although a recent census had indicated that there were 40 species and over 8000 breeds of domestic animals; for the purpose of conservation biology the diversity between and within breeds rather than species is regarded to be of crucial importance. This domestic animal genetic diversity has developed through three main evolutionary events, from speciation (about 3 million years ago) through domestication (about 12,000 years ago) to specialised breeding (starting about 200 years ago). These events and their impacts on global animal genetic resources have been well documented in the literature. The key importance of global domestic animal resources in terms of economic, scientific and cultural heritage has also been quantified. In spite of the importance, there have been a growing number of reports on the alarming erosion of domestic animal genetic

resources. This erosion of animal genetic resources is happening in spite of several global conservation initiatives designed to mitigate it. In this review, we discuss these conservation interventions and highlight their strengths and weaknesses. However, pivotal to the success of these conservation initiatives is the reliability of the genetic assignment of individual members within a target breed. Finally, we discuss the prospect of using improved breed identification methodologies to develop a reliable breed specific molecular identification tool that is easily applicable to populations of livestock breeds in various ecosystems. These identification tools, when developed, will not only facilitate the regular monitoring of threatened or endangered breed populations, but also enhance the development of more efficient and sustainable livestock production systems.

Key words: Conservation, Diversity, Genetic Resources, Global Livestock, FAO, Molecular Techniques, Threats, Breeds.

CONTENTS

## I. INTRODUCTION

A significant amount of genetic variation present in wild animal lineages prior to domestication was conserved during the domestication process, and persisted within their respective domesticates (Dobney & Larson, 2006). Over the 12,000 years since farm animals were first domesticated, their genetic make-up has undergone adaptation due to both natural (speciation) and artificial (domestication/ breeding) selection pressures exerted by their specific environments and human activity respectively (Banik, Pankaj & Naskar, 2015; Hoffmann & Scherf, 2005; Jensen, 2006; Mignon-Grasteau, Boissy, Bouix *et al.*, 2005; Morris, 2006; Naskar, Gowane & Chopra, 2015; Price, 1999; Vigne, 2011; Zeder, Emshwiller, Smith *et al.*, 2006). These selection pressures have culminated in the development of a rich global domestic animal diversity with thousands of breeds (Ajmone-Marsan & Globaldiv Consortium, 2010; Groeneveld, Lenstra, Eding *et al.*, 2010). Each of these breeds is characterised by their unique morphology and productivity related to specific environmental and applied farming conditions (Lopes, Mendonça, Rojer *et al.*, 2015; Shand, 1997). A livestock breed can be generally defined as either a homogenous group with unique and identifiable phenotypic features that distinguish it from other sub groups within the same species, or a homogenous group for which geographical isolation from other groups of the same species has resulted in their acceptance as unique entities (FAO, 2000; Rege, 2003). Recently a more refined definition of a breed concept to encompass the history of the livestock was proposed by Felius, Theunissen and Lenstra (2014) and Tixier-Boichard (2014). This proposal will conform to current practical reality as not all breeds by definition actually represent unique genetic resources. Breeds can therefore be regarded as the unit

of management for livestock instead of unit of conservation so as to make it more instrumental for conservation purposes (Felius *et al.*, 2014; Groeneveld *et al.*, 2010).

A recent report on livestock breed diversity stated that there were 7202 local breeds (breeds found in only one country), 509 regional trans boundary breeds (breeds found in different countries within one region) and 551 international trans boundary breeds (breeds found in different countries in different continents) (FAO, 2013). These breed classifications comprised the seven main mammalian livestock species (sheep, goats, cattle, pigs, buffalo, horses, and asses/donkeys), four main avian livestock species (chicken, turkeys, ducks, and geese) and eight minor livestock species (alpacas, yaks, llamas, camels, elephants, musk oxen, and guinea pigs). However, since the concept of selective breeding emerged in the last 200 years, and subsequently, through more intensive selection in the last few decades, domestic animal diversity has been under sustained threat of significant erosion (Ajmone-Marsan *et al.*, 2010; Köhler-Rollefson, 1997). In 2012, an analysis of data from 182 countries at the Global Databank for farm animal genetic resources revealed that 8% of farm animal breeds could already be considered extinct, 22% were at varying degrees of extinction risk, and the risk status of 34% was unknown (FAO, 2013). The report indicated that only approximately 36% of global farm animal genetic resources were not at any risk of immediate extinction.

This growing threat to the world's animal genetic resources was recognised by the Food and Agriculture Organisation of the United Nations (UN) as an emerging global challenge, and this recognition has led to the ratification by 109 countries, in 2007, of the Interlaken Declaration on world animal genetic resources (Rischkowsky, Pilling, Food *et al.*, 2007). The Interlaken Declaration was the first ever such global action plan specifically aimed at conserving our current animal genetic resources. The declaration called for urgent and prompt measures to be undertaken to mitigate the risk of large scale loss of defined breeds in the face of challenges such as increasing human population, climate change and emerging diseases. It was also envisaged that such intervention,

when successful, would also make a significant contribution to millennium development goals 1 and 7:- eradication of extreme poverty and hunger, and ensuring environmental sustainability, respectively. The millennium development goals (or agenda) are a blue-print of eight goals referred to as the UN Millennium declaration, which was commissioned by the UN general assembly in September, 2000 (United Nations, 2000). The objective of the declaration is to galvanize unprecedented efforts from all member countries to reverse the poverty, hunger and disease affecting billions of people around the world within a 15 year time frame. Despite the historic breakthrough at the Interlaken Summit, not much progress has been made so far, especially in developing countries, due to several factors, the most prominent being a general lack of technical capacity and financial resources (FAO, 2007). The Domestic animal diversity information system (DAD-IS) is an information and communication tool that was set up to coordinate management strategies developed for domestic animal diversity at global, regional and national levels. This system has challenges, especially regarding the quality of the entries from developing countries (Tixier-Boichard, 2014). Most of the data submitted, especially from Africa, requires regular updating to make it relevant to the current situation. For example 48% and 53% of mammalian and avian breeds recorded in DAD-IS were found to lack sufficient demographic information necessary for the assessment of their respective risk status. Furthermore, 87% of entries regarding breed demographics were found to be based on a survey or census, thus presenting a significant limitation and might be unreliable. In recognition of these and other shortcomings in attempts at addressing the global animal genetic resource erosion issues, the European Union commissioned another three year global programme named 'The GLOBALDIV Project' (Ajmone-Marsan *et al.*, 2010). The GLOBALDIV project also known as "global view of livestock biodiversity and conservation" had representations from the FAO of UN, the International Livestock Research Institute (ILRI), the International Atomic Energy Agency (IAEA), and 34 individual international researchers from key institutions that are working in areas related to the characterisation of farm animal genetic resources. The main aim of this project is to integrate and disseminate the experience of past, large

scale, biodiversity projects and to review the main drivers of biodiversity loss, and to implement strategies for the conservation of farm animal genetic diversity. Notable among the recommendations of the GLOBADIV project is the need for amalgamation of the disciplines of genetics, socioeconomics and geographic information science for efficient valuation of domestic animal genetic resources. Currently, improved geo-referencing methods, for example global positioning systems (GPS), are being used as part of a range of measures to provide better production environment descriptors (Groeneveld *et al.*, 2010). However, because of the dynamic nature of domestic livestock diversity, it is now obvious that more innovative interventions are required to provide precise information on breed structure and status and effectively halt the rapid loss of global livestock genetic diversity. For any livestock breed considered to be at risk, it is recommended that the monitoring of population trends in terms of population size and structure must be carried out at least once per generation. The development of breed specific identification tools for each characterised livestock breed will not only facilitate this process of regular monitoring of population trends and demographics, but also promote conservation.

This review summarizes our knowledge of; (i) the key importance of domestic animal genetic resources, (ii) the threats to this resource diversity, (iii) the current status of domestic animal genetic resources, and (iv) conservation methods, with specific emphasis on a molecular genetics approach. We conclude with an assessment of the potential development and use of reliable breed identification tools for livestock breeds for enhancing modern conservation biology studies and preservation of livestock breed diversity.

## II. KEY SCIENTIFIC, CULTURAL AND ECONOMIC IMPORTANCE OF GLOBAL LIVESTOCK GENETIC RESOURCES

Domestic livestock are known to directly provide food and livelihoods to more than 90% of the 1.97 billion people who live on less than one US Dollar a day (Anderson, 2003; ILRI, 2009). With a total global asset value of US$ 1.4 trillion dollars, domestic livestock is reported to contribute 33% and

17% to global protein and kilocalorie consumption, respectively (Herrero, Thornton, Gerber *et al.*, 2009). In many developing countries, apart from the provision of food and income, livestock transactions also develop and foster meaningful and emotional social relationships between and among communities (McCorkle & James, 1996). The so called minor livestock species, although fewer in population number and distribution, are typically of critical importance in terms of cultural heritage and for the livelihood of their owners (McCorkle *et al.*, 1996; York & Mancus, 2013). For instance draught animal power plays essential role in the livelihoods of marginal communities in many developing countries in Asia, sub Saharan Africa, and Latin America (Barrett, 1992; Lawrence & Pearson, 2002; Teweldmehidin & Conroy, 2010). In addition to these traditional important uses, several species of animals are now used as models in toxicology studies to ascertain the hazard level to human of prospective drugs (Olson, Betton, Robinson *et al.*, 2000). For example the miniature pig was identified as the ideal non-human primate model for chromosomal abnormalities, skin cell therapy and neural stem cell studies (Vodička, Smetana, Dvořánková *et al.*, 2005). Also a strain of rabbit referred to as Watanabe heritable hyperlipidemic was found to be a good model for the study of human myocardial infarction (Shiomi, Ito, Yamada *et al.*, 2003). It has been recommended that comparative medicine, which entails disease studies across animals and human species, holds the key to efficient prevention and control strategies for many zoonotic diseases (Kahn, 2006). Livestock diversity should not only be considered on the basis of global food security, but also as having critical cultural, economic and scientific importance, both currently and into the future.

## III. THREATS TO GLOBAL LIVESTOCK GENETIC RESOURCES

The global domestic animal or livestock genetic resources (AnGR) are defined as the sum total of animal species, breeds and strains that currently are, or may be of, future economic, scientific and cultural heritage importance to humans. For the purpose of conservation it is usually breed diversity rather than species diversity that is of greater importance (Philipsson, Zonabend, Bett *et al.*, 2011). According to the latest report by the commission on animal genetic resources the percentage of

local livestock breeds considered to be at the risk of extinction increased by two percentage points between 2010 and 2012 (FAO, 2013). This outlook on the prevailing extinction rate of livestock, although alarming, is likely to be an under-estimation of the actual situation, especially in relation to estimates for developing regions of the world such as sub-Saharan Africa (Rege & Gibson, 2003). The loss of livestock genetic diversity reduces the range of opportunities available to confront the challenges of unpredictable future events, such as climate change, social change, disease epidemics, selection errors, and many others (Anderson, 2003; Anderson & Centonze, 2007).

There is a wide spectrum of interrelated man-made and natural factors that pose varying levels of threats to global AnGR (Philipsson *et al.*, 2011; Rege *et al.*, 2003). The factors that are responsible for the erosion of genetic diversity are often a function of the size of the population under consideration (Barbato, Orozco-terWengel, Tapio *et al.*, 2015). Generally, the smaller a livestock population, the greater is its vulnerability to extinction (Biscarini, Nicolazzi, Stella *et al.*, 2015; Henson, 1992; Ramstad, Woody, Sage *et al.*, 2004). Human factors offer the greatest threat to global livestock diversity (Biscarini *et al.*, 2015; Frankham, 1995). The human factors include, but are not limited to; intensive selective breeding, over exploitation, political instability and wars (Goe & Stranzinger, 2002), indiscriminate crossbreeding (Alvarez, Traoré, Tamboura *et al.*, 2009; Wollny, 2003) and general neglect or lack of breeding programmes (Rege, Marshall, Notenbaert *et al.*, 2011; Wollny, 2003). Interestingly, these human factors vary across both developed and developing regions of the world. In the developed regions, the threat to livestock diversity is mostly associated with overexploitation such as specialised breeding in response to dynamic socioeconomic pressures (Groeneveld *et al.*, 2010). This trend is also expedited partially by often misguided or inappropriate application of advanced molecular biology technologies (Tisdell, 2003). Conversely in the developing countries, the main factors are a general neglect of livestock and or poorly structured breeding programmes driven in part by lack of technical knowledge and financial resources (Alvarez *et al.*, 2009; Biscarini *et al.*, 2015; Philipsson *et al.*, 2011). In the face of this clear dimorphism, what is of utmost importance are the measures necessary to minimise the “Swanson dominance effect” from

happening (Tisdell, 2003). The Swanson dominance effect refers to a phenomenon in which the choices made by the earliest developing societies influence the later pattern of development in another. There have been reports of livestock keepers in parts sub-Saharan Africa abandoning their locally adapted breeds in favour of specialised potentially highly productive, but non-adapted exotic breeds, thereby leading to a decline in diversity (Groeneveld *et al.*, 2010; Wollny, 2003). Nonetheless, regardless of the region of the world, general increases in human population tend to impact negatively on livestock diversity.

Natural events that have commonly been cited as major causes of erosion of livestock genetic resources include; Tsunamis, Earthquakes, Hurricanes, droughts, disease epidemics, famine and floods (Prentice & Anzar, 2011). In the past two or more decades, climate change has emerged as a higher-level driving force for reduction in AnGR (Nardone, Ronchi, Lacetera *et al.*, 2010; Thornton, van de Steeg, Notenbaert *et al.*, 2009). Many reports have described the expected impact of climate change on livestock production systems and diversity (Banik *et al.*, 2015; Herrero *et al.*, 2009; Hoffmann, 2010; Kantanen, Løvendahl, Strandberg *et al.*, 2015; McMichael, Powles, Butler *et al.*, 2007; Naskar *et al.*, 2015). This is mainly because of the direct and indirect implication of climate change on both the frequencies and intensities of most of the causative factors for genetic erosion mentioned previously (Naskar *et al.*, 2015). The irony, however, is that livestock contributes significantly to climate change, as they contribute about a fifth of global greenhouse gas emissions (Garnett, 2009; Gavrilova, Jonas, Erb *et al.*, 2010; McMichael *et al.*, 2007; Shields & Orme-Evans, 2015).

Natural and human-made evolutionary forces either directly or indirectly can cause a reduction in the effective population size ($N_e$) of a livestock breeding population. Therefore, the genetic variability of subsequent populations is drastically reduced because it is derived from the genetic constitution of the few survivors remaining from the original population (Allendorf, 1986). In population genetic studies these reductions in population size are referred to bottlenecks. A

population that passes through a bottleneck loses alleles and usually shows reduced average heterozygosity (Allendorf, 1986; Nei, Maruyama & Chakraborty, 1975), but could also lead to temporarily, an increased in heterozygosity if more rare alleles are lost in the process (Hundertmark & Van Daele, 2010; Luikart & Cornuet, 1998). Regardless of the cause of a bottleneck, it may take several generations to restore the original level of heterozygosity through the occurrence of new mutations (Chakraborty & Nei, 1977). The impact of a bottleneck is logically more profound on small breeding populations because the originally available pool of genetic diversity is smaller, and is hence more severely depleted in fewer generations. In population genetic studies a bottleneck effect is referred to as a founder effect if it is associated with the founding of a new population (Dlugosch & Parker, 2008; Ramstad *et al.*, 2004; Templeton, 1980). Random events such as founder and bottleneck effects that imperfectly eliminate genes and reduce variability within a population are described as genetic drift (Newman & Pilson, 1997; Ramstad *et al.*, 2004). Reduction in heterozygosity in a livestock population can be associated with decline in fitness of individual members, as is often the case in wild populations (Worley, Collet, Spurgin *et al.*, 2010). This is because within small populations, the rate of inbreeding is much higher and consequently there is higher likelihood of the expression of deleterious recessives in a homozygous state. The expression of deleterious alleles has adverse effects on the livestock population often presenting as reduced production, reproduction and survival (Dlugosch *et al.*, 2008; Lacy, 1997). Frankham (1995) and Lacy (1997) have described the positive correlation between inbreeding and risk of extinction. The effective population size model takes into account important population variables such as age and structure, inbreeding rates, genetic drift, genetic diversity and sex ratio. For example, a population of four males and four females constitutes the same effective population size as that of 100 females and only two males (Henson, 1992).

In a breed regeneration programme, the effective population size can be enhanced by equalizing the male to female ratio, and standardizing litter size and longevity within the breeding population, so as to ensure that each animal contributes equally to the next generation. Therefore the effective

population size is the preferred indicator of livestock conservation risk status (Dlugosch *et al.*, 2008; Nei *et al.*, 1975). However, it is apparent that the estimation of the effective population size and, subsequent determination of its conservation status for a given breed is limited by the lack of availability of a reliable breed identification tool for any specific breed.

## IV. ASSESSMENT OF LIVESTOCK GENETIC DIVERSITY AND CONSERVATION STATUS

In order to sustainably manage livestock genetic resources a comprehensive knowledge of diversity within and between breed populations is required (Groeneveld *et al.*, 2010). A major step towards standardising the assessment criteria for livestock breed conservation status was the establishment of a universal classification framework by the FAO for categorizing risk status. The current classification of livestock conservation risk status contains seven categories, namely; extinct, critical, critical-maintained, endangered, endangered-maintained, not at risk, and unknown (FAO, 2013). Regular assessment of genetic conservation status of livestock is of fundamental importance to prevent genetic erosion and to preserve diversity. Key to achieving an effective assessment of livestock conservation status is a reliable mode of identification of members of a target breed. There are two broad methods for identifying individual members of a livestock breed, and their merits and demerits have been discussed thoroughly (Agaviezor, Peters, Adefenwa *et al.*, 2012; Ashley & Dow, 1994; Birteeb, Peters, Yakubu *et al.*, 2012). These methods comprise phenotypic and molecular identification techniques. Traditionally phenotypic identification has been used to identify the breed of an individual in livestock genetic diversity studies. The phenotypic parameters usually used comprise; physical features (e.g. Shape of horn, ears, body measurements, colour etc.), production traits (e.g. growth parameters), reproductive traits (e.g. fecundity) and survival traits (e.g. Disease resistance, drought resistance) (Brinks, Clark, Kieffer *et al.*, 1964; Gwakisa, Kemp & Teale, 1994; Reverter, Johnston, Ferguson *et al.*, 2003). These methods are extensively used because they are inexpensive and often do not require the use of any sophisticated equipment. However, the major

disadvantage is that the genetic diversity is observed only at the phenotypic level and this does not always correspond to actual diversity at the DNA level (Felius *et al.*, 2014).

It is possible to find different phenotypes with similar genotypes, typically due to genotype-environment interactions, for example as observed in Brazilian sheep Breeds (Paiva, Faria, Silvério *et al.*, 2005) and Egyptian sheep breeds (Ali, 2003). Similar phenotypes with different genotypes also occur, as observed in West African Djallonke sheep (Alvarez, Capote, Traoré *et al.*, 2012; Alvarez *et al.*, 2009; Wafula, Jianlin, Sangare *et al.*, 2005). As a result, the use of molecular tools in many assessment studies of genetic diversity in different regions of the world revealed varying degrees of unexpected introgression and admixture in livestock populations. Some of the reported studies include; the Djallonke sheep breed of sub-Saharan Africa (Alvarez *et al.*, 2009; Wafula *et al.*, 2005), Herdwick sheep of the United Kingdom (Bowles, Carson & Isaac, 2014) and alpaca and llama of Latin America (Kadwell, Fernandez, Stanley *et al.*, 2001). This obvious shortcoming has rendered the use of the phenotypic method alone unreliable for determination of livestock breeds for the purpose of genetic diversity studies.

In livestock genetic diversity studies, the molecular method for determining breed identity entails two main approaches based upon either protein markers or DNA markers (Ferguson, Taggart, Prodöhl *et al.*, 1995; McMahon, Teeling & Höglund, 2014). Protein markers, also referred to as allozymes, are based on the characteristic polymorphism of the blood group systems, leucocyte antigens and enzymes (Dodgson, Cheng & Okimoto, 1997). This molecular method employs these protein markers to estimate genetic variability in livestock populations as well as phylogenetic relationships between breeds (Pepin & Nguyen, 1994; Witko-Sarsat, Friedlander, Capeillere-Blandin *et al.*, 1996). Although better than the phenotypic method, the use of protein markers is too expensive for large number of loci, and lacks the power to resolve differences between closely related breeds, because of limits of detection of genetic variation (Engel, Linn, Taylor *et al.*, 1996; Ferguson *et al.*, 1995; Toro, Fernández & Caballero, 2009). The use of DNA markers is the most

reliable molecular method for assessment of genetic diversity (Liu & Cordes, 2004). Nuclear and mitochondrial DNA marker analyses have revealed detailed information on many domestication events, such as their timing and location (Bruford, Bradley & Luikart, 2003; Zhao, Zheng, Dong *et al.*, 2013). DNA marker analyses provide an added opportunity for investigating the genetic composition of both extinct and endangered breeds without destructive sampling. There are seven principal DNA marker techniques commonly used for livestock diversity studies (Sunnucks, 2000). The seven DNA marker principal techniques have been discussed thoroughly and their advantages and disadvantages are well documented by many authors. These techniques are; Restriction Fragment length Polymorphism (RFLP) (Beckmann & Soller, 1983; Beckmann & Soller, 1986; Thurston, Siggins, Mileham *et al.*, 2002), Mitochondrial DNA barcoding (mtDNA) (Avise, Arnold, Ball *et al.*, 1987; Avise & Ellis, 1986; Harrison, 1989; Kocher, Thomas, Meyer *et al.*, 1989; Zhang & Hewitt, 1996), Random Amplified Polymorphic DNA (RAPD) (Ali, Huang, Qin *et al.*, 2004; Dodgson *et al.*, 1997; Koh, Lim, Chua *et al.*, 1998; Levin, Crittenden & Dodgson, 1993), Amplified fragment Length Polymorphism technique(AFLP) (Blears, De Grandis, Lee *et al.*, 1998; Parsons & Shaw, 2001; Savelkoul, Aarts, De Haas *et al.*, 1999), Y-chromosome technique (Bruford *et al.*, 2003; Zeder *et al.*, 2006), and the two most popular DNA techniques are Variable Number of Tandem Repeats (Minisatellite and Microsatellite markers) (Chistiakov, Hellemans & Volckaert, 2006; Fan & Chu, 2007; Lopes *et al.*, 2015; Zane, Bargelloni & Patarnello, 2002) and Single Nucleotide Polymorphism (SNP) based techniques (Andersson & Georges, 2004; Liu *et al.*, 2004; McMahon *et al.*, 2014; Morin, Luikart & Wayne, 2004; Vignal, Milan, SanCristobal *et al.*, 2002).

The advancement of DNA technologies during the past three decades, and particularly since 2007 when high throughput next generation sequencing became readily available, is revolutionising livestock population genetics studies (Helyar, Hemmer-Hansen, Bekkevold *et al.*, 2011; Schlötterer, Kofler, Versace *et al.*, 2014). This revolution is expedited by the concomitant advancement in bioinformatics tools and pipelines (Kofler, Nolte & Schlötterer, 2015). DNA markers have been used not only for diversity studies but also for molecular characterisation of numerous livestock breeds

worldwide (Agaviezor *et al.*, 2012; Al-Atiyat, Salameh & Tabbaa, 2014; Alvarez *et al.*, 2012; Bowles *et al.*, 2014). The dramatic reduction in the cost of use of DNA markers has facilitated their greater use by researchers. For the purpose of this review the Variable Number Tandem Repeats (VNTR) and the Single Nucleotide Polymorphism (SNP) techniques will be discussed in greater detail because of their wider application compared with the other molecular markers types. AFLP and RAPD markers are both bi-allelic, but dominant in nature, and hence are less informative and also have low reproducibility compared to the other markers (Vignal *et al.*, 2002). These characteristics have rendered them less popular for most animal based molecular genetic studies. RFLP markers are bi-allelic and co-dominant, and were famously used in the first large scale mapping of the human genome. However, they have now been superseded by multi-allelic and more informative microsatellite markers in both animal and human genome studies which, in turn have been largely superseded by single nucleotide polymorphism (SNP) arrays. MtDNA along with microsatellite markers were once a popular molecular genetic techniques of choice for evolutionary and ecological studies, however the molecular information provided by mtDNA markers is limited to only maternally inherited loci (Morin *et al.*, 2004). The use of mtDNA techniques, in combination with archaeological data, have provided precise information on most of the important centres of domestication for the main livestock species around the world (Bruford *et al.*, 2003; GLOBALDIV CONSORTIUM, 2010; Guo, Savolainen, Su *et al.*, 2006; Zeder *et al.*, 2006). Similarly limited, the use of Y-chromosomal haplotype markers elucidates specific molecular information only on paternal inherited traits (Luikart, Fernández, Mashkour *et al.*, 2006).

### IV 1. VARIABLE NUMBER OF TANDEM REPEATS (VNTRS)

The application of VNTRs for assessment of genetic variation, sub structuring and hybridization in natural populations has been well reviewed in great detail by many investigators (Bruford & Wayne, 1993; Chistiakov *et al.*, 2006; Fan *et al.*, 2007; Sunnucks, 2000). The VNTR technique is based on the abundance of tandem repeats of simple sequences of nucleotides throughout the eukaryotic

genome (Takezaki & Nei, 2008). These VNTRs have been categorised into minisatellites and microsatellites according to the number of nucleotides per motif of repeats. VNTRs of between 1 to 6 nucleotide base pair units are referred to as microsatellites (Ashley *et al.*, 1994; Chistiakov *et al.*, 2006; Fan *et al.*, 2007), whereas a range of between 10 to 60 nucleotide base pair units is regarded as a minisatellite (Ashley *et al.*, 1994; Wasko & Galetti, 2003). Whereas minisatellites are concentrated towards the telomere of chromosomes, microsatellites are randomly distributed in chromosomes. Microsatellite markers are highly polymorphic, codominant markers of relatively small size, and hence are more amenable to PCR typing than are minisatellites (Zane *et al.*, 2002). Also, in comparison to the RFLP and RAPD techniques, the genetic basis of variability is readily apparent for microsatellites. Most microsatellites are located in non-coding regions of the genomes, and very few are located within the coding regions (Chistiakov *et al.*, 2006). Generally, microsatellite primers developed for one species of livestock are broadly applicable to other closely related species. For example, microsatellite markers developed for studies in bovine species are applicable for caprine and ovine species (Engel *et al.*, 1996). The reason for this wide applicability of microsatellite markers stems from the fact that they are conserved across wide taxa of related species. This versatility has led to the popularity of microsatellite maps for economically important livestock species (Sunnucks, 2000).

Microsatellites have been used in linkage mapping in diverse organisms, for example in the bovine genome (Barendse, Vaiman, Kemp *et al.*, 1997), porcine genome (Rohrer, Alexander, Keele *et al.*, 1994), human genome (Dib, Fauré, Fizames *et al.*, 1996), and ovine genome (Maddox, Davies, Crawford *et al.*, 2001). Microsatellite have also been employed for the identification of Quantitative Trait Loci (QTL) in major livestock species, for example, carcass composition and growth rate in cattle (Casas, Shackelford, Keele *et al.*, 2000), back fat thickness and intramuscular fat in pigs (Rohrer & Keele, 1998) and intestinal parasitic infection in sheep (Davies, Stear, Benothman *et al.*, 2006). Other population genetics studies accomplished with microsatellite markers include; determination of evolutionary relationships (Buchanan, Adams, Littlejohn *et al.*, 1994), estimation of pedigree errors

(Visscher, Woolliams, Smith *et al.*, 2002), determination of genetic diversity among livestock populations (Alvarez *et al.*, 2012; Alvarez *et al.*, 2009; Marletta, Tupac-Yupanqui, Bordonaro *et al.*, 2006; Medugorac, Veit-Kensch, Ramljak *et al.*, 2011; Wafula *et al.*, 2005), and last but not least, genetic distance between livestock breeds (Alvarez *et al.*, 2012; Buchanan *et al.*, 1994; Vanhala, Tuiskula-Haavisto, Elo *et al.*, 1998). The genetic distance of individuals within a livestock population indicate the suitability of an individual for conservation purposes. Individuals within the same breed with the widest differences in genetic distances are deemed most suitable candidates for conservation programmes. The estimates of genetic distances are also relevant for the determination of divergence time and construction of phylogenies (Takezaki & Nei, 1996).

## IV 2. SINGLE NUCLEOTIDE POLYMORPHISM (SNP) MARKERS

The growing importance of SNP marker applications in molecular genetics has been reviewed in detail by Barbato *et al.* (2015), Broxham (2015), Goddard and Hayes (2009), Vignal *et al.* (2002), Hamblin, Warburton and Buckler (2007) and Morin *et al.* (2004). Prior to the use of SNP markers, microsatellites were the most popular and efficient technique for genetic diversity investigation, not only in livestock but also in humans as well. SNPs represent a location within a DNA sequence for which more than one nucleotide type is present within a given population (Morin *et al.*, 2004). In a strict molecular sense, SNPs are base substitutions within nucleotide sequences, and the very high density of their occurrence in the genomes of eukaryotes, including livestock, has been of great significance in population genomics studies (Goddard *et al.*, 2009; Vignal *et al.*, 2002). Although SNPs are bi-allelic (sometimes tri-allelic or quadri-allelic), co-dominant molecular markers, their high density permits, more than any other technique, very detailed information to be elucidated on genome dynamics within a study population (Hamblin *et al.*, 2007; Morin *et al.*, 2004). Furthermore, they provide deeper insight than microsatellites with respect to linkage disequilibrium and haplotype diversity, pedigree information as well as past demographic events, such as bottlenecks within a target population (Clarke, Henry, Dodds *et al.*, 2014; Helyar *et al.*, 2011; Morin *et al.*, 2004;

Vignal *et al.*, 2002). These features of SNP markers, coupled with a relatively low error rate, are opening opportunities for wider applications of SNP markers in understanding of livestock genetic architecture, such as precise identification of genomic regions that control traits of economic and survival importance (Kohn, Murphy, Ostrander *et al.*, 2006; Matukumalli, Lawley, Schnabel *et al.*, 2009) and ultimately genomic selection (Clarke *et al.*, 2014; Goddard *et al.*, 2009). These advances in genetic marker application for use in population genetic studies will not only enhance the development of improved livestock production systems, but most importantly will facilitate the development of efficient conservation strategies. High density SNP chips have been used to accurately and correctly cluster sheep breeds from different continents (Kijas, Townley, Dalrymple *et al.*, 2009), and more recently for the effective management of highly endangered breeds of small cattle in the Balkans (Broxham, 2015). A study involving typing 47,594 snps from pigs derived from domestic and wild populations on the Iberian Peninsula showed that principal component analysis could assign animals to a specific population (Herrero-Medrano, Megens, Groenen *et al.*, 2013). Furthermore, effective population size, past effective population size and runs of homozygosity could be determined from the data. This shows that snp chips can be used to determine relationships between populations and levels of inbreeding.

## V. CONSERVATION METHODS FOR DOMESTIC ANIMAL GENETIC RESOURCES

Conservation of AnGR basically comprises all the management practices carried out to preserve the pool of genetic diversity of livestock for the purpose of meeting the current and future needs of humans (Rege *et al.*, 2003). The relevance of conservation of AnGR has been thoroughly discussed by many authors, from several different perspectives, for example; economic evaluation as a basis for AnGR conservation decisions (Drucker, Gomez & Anderson, 2001), the role of cryopreservation, reproductive technologies and genetic resource banks for AnGR conservation strategies (Hiemstra, van der Lende & Woelders, 2006b; Holt & Pickard, 1999; Mara, Casu, Carta *et al.*, 2013), information on population kinships as a basis for AnGR conservation decisions (Eding & Meuwissen, 2001) and

the challenge of conserving indigenous AnGR diversity (Mendelsohn, 2003). Each breed of livestock consists of unique sets of genes resulting from evolutionary events and diverse selection pressures imposed by the environment combined with the activities of humans over time. It is therefore difficult, if not impossible, to replace lost breeds of livestock, because those unique evolutionary processes cannot be re-created. There has been a general consensus on three critical approaches regarding the conservation of domestic livestock breeds, and these are; sustainable utilization of available livestock breeds, appropriate diversity based improvement strategies for livestock breeds, and development of appropriate assessment and preservation strategies (FAO, 2000; Hammond, 1999; Koehler-Rollefson & Meyer, 2014; Notter, Mariante & Sheng, 1994; Thornton, Boone, Galvin *et al.*, 2007). In addition to these approaches, the FAO has also recommended the regular monitoring of livestock breed conservation status. Currently the two main methods of AnGR conservation applied are the *in situ* and the *ex situ* methods. The applicability of both conversation methods, and their respective merits and demerits has been reported extensively (Boettcher, Tixier-Boichard, Toro *et al.*, 2010; Hammond, 1994; Henson, 1992; Mara *et al.*, 2013; Rege *et al.*, 2003). *In situ* conservation can best be described as the sustainable breeding of an endangered livestock breed in their normal adaptive production environment, or as close to it as practically possible, to conserve genetic diversity over a long time (Andrabi & Maxwell, 2007; Henson, 1992). Notable features of *in situ* conservation therefore include selection and mating programmes that enhance genetic variation within the target group, as well as management of the ecosystem to sustain their production. The basic requirements for *in situ* conservation programmes are generally readily available and affordable globally. There is a distinct difference between developed and developing countries regarding the minimum number of individuals required to commence an *in situ* programme. This is typically due to the general differences in the efficiency of management of their respective livestock production systems. There are different minimum numbers of animals reported to be required for *in situ* conservation. For example, whereas the minimum number for major livestock breeds (i.e. cattle, sheep, goats, pigs) required for *in situ* conservation is 100-1000 breeding females in developed

countries, no fewer than 5000 breeding females is recommended for developing countries (Signorello & Pappalardo, 2003). Simon (1999) reported 500 breeding females for pigs and goats, 750 for cattle, and 1500 for sheep for European breeds. It has been recommended that, ideally, for unrelated animals a minimum of 25 males and 50 females is sufficient to commence an *in situ* conservation programme, because the possible loss of genetic variability is estimated to be less than 1% per generation (Henson, 1992; Mara *et al.*, 2013).

There are a number of flagships *in situ* conservation programmes in place to conserve and improve some disease resistant breeds of livestock in some African countries, for example, Ndama cattle in the republic of Guinea, Djallonke sheep in Ghana, and Tswana sheep in Botswana (Henson, 1992; Kosgey & Okeyo, 2007; Yapi-Gnaoré, Dagnogo & Oya, 2003). The unique advantage of the *in situ* conservation method is that the target livestock continues to be utilized in the process. However, the danger is that the target livestock remains susceptible to uncertain demographic threats such as natural disasters and disease epidemics. The *ex situ* livestock conservation method is the preservation of endangered livestock outside their normal production systems (Henson, 1992; Hiemstra, Drucker, Tvedt *et al.*, 2006a). This method is normally applied to target groups that are faced with imminent extinction, and hence requires the use of high level expertise and technology. The three main *ex situ* methods are cryopreservation, farm park conservation, and breed pools or composite preservation.

Cryopreservation, also referred to as *in vitro ex situ* is undoubtedly the most popular of the *ex situ* approaches to conservation of AnGR (Hiemstra *et al.*, 2006a). This approach involves the cryopreservation of eggs, semen and or embryos of endangered or threatened animals in genome banks for use in managing diversity or regenerating the population decades or even centuries later (Chen, Zhang & Yu, 2008; Hanks, 2001; Russo, Martelli, Mauro *et al.*, 2007; Xiao-Yong, Shu-Jun, Run-Zi *et al.*, 2008). Cryogenic storage of carefully evaluated genetic material from a target breed population is also seen as an insurance policy against future loss. The merits and demerits of using

these approaches have been previously discussed (Boettcher *et al.*, 2010; Munro & Adams, 1991; Philipsson, Rege & Okeyo, 2006; Pintado & Hourcade, 2011; Ruane & Sonnino, 2011). The application of cryopreservation formerly depended only on assisted reproductive techniques such as artificial insemination and embryo transfer technologies. However, recent advances in reproductive biotechnologies including semen sexing, embryo micromanipulation and *in vitro* fertilization have the potential to revolutionise the livestock cryopreservation approach (O'Brien, Steinman & Robeck, 2009; Prentice *et al.*, 2011). The cryopreserved genetic materials are shielded from the influence of the unfavourable environmental conditions in existence in the normal production ecosystems. Regeneration of a breed through only preserved semen requires a number of back crosses to be done. However, the entire genetic composition of the original breed is not recoverable with only cryopreserved semen (Andrabi *et al.*, 2007). In practice, the *in situ* and *ex situ* conservation methods are not mutually exclusive because the cryopreservation approach can be used to complement the *in situ* method to achieve better regeneration of endangered populations. A range of combinations of *in situ* and *ex situ* conservation methods are being applied in a now popular integrated conservation approach (de Souza, Batista, Melo *et al.*, 2011; Hiemstra *et al.*, 2006a). It has been recommended that a stock of cryopreserved semen from 25 unrelated sires is sufficient to provide a reasonable diversity to an endangered population (Bruns & Glodek, 1999; Mara *et al.*, 2013).

The farm park *ex situ* conservation approach is similar to the *in situ* conservation approach, except that the targeted breeds are preserved outside their normal production environment in a specialised institutional setting, also referred to as an Ark-farm (Simon, 1999). Farm park animals are usually also subjected to more stringent management regimes to conserve natural levels of genetic variability within each species(Chesser, Smith & Brisbin, 1980). A notable feature of the Farm Park approach is its popularity in attracting tourists, and hence creating awareness of the need to conserve endangered animals. The Cotswold farm park in the UK is an example where rare breeds of sheep, goats, cattle, pigs and horses are being conserved, and it attracts more than 100,000 visitors yearly (Henson, 1992). The breed pool preservation programme is unique in the sense that it

involves the breeding together of a pool of two to four rare breeds with similar characteristics, and subsequently managing their offspring to conserve genetic variation (Henson, 1992). It is, however, recommended that the breed characteristics of each of the rare breeds is well ascertained prior to commencing a breed pool programme (Santos, Affonso, Diniz *et al.*, 2013). This method is particularly suitable for genes that control obvious morphological traits and extreme quantitative traits such as coat colour and prolificacy, respectively. Although this approach conserves useful genes from the pool, individual breeds are lost in the process. An example of the breed pool approach is the conservation programme of four rare desert goat breeds, in the north eastern part of Brazil (Henson, 1992).

Given that no single conservation method is capable of solving the myriad of challenges of domestic animal genetic resource erosion, an integrated conservation approach has been advocated to provide more efficiency (Rege *et al.*, 2003).

## VI. MODELLING: THE WAY FORWARD

The successful domestication of animals represents a pivotal historic event in the cultural and demographic development of humans. The importance of global domestic livestock diversity to human wellbeing is now well-appreciated. This is evident by the globally coordinated efforts directed at halting the decline in AnGR as well as the sustainable utilisation of available livestock resources as discussed in this review. These global initiatives have yielded several interventions which are being implemented at the international, regional and local level (Ajmone-Marsan *et al.*, 2010; FAO, 2013). The main global body is the DAD-IS that coordinates regional bodies, for example EFABIS (European farm Animal Biodiversity information System) and DAGRIS (Domestic Animal Genetic Resource information System) for European and African regions respectively. The regional bodies in turn coordinate the local or national bodies, which essentially are the individual member states of the FAO of the United Nations. These efforts are being supplemented by the activities of other important organisations, prominent members including; the GLOBALDIV, the International Society

for Animal Genetics (ISAG), the SAVE foundation and several livestock breed societies worldwide (Broxham, 2015). Some notable progress has been made towards reducing the rate of erosion of global AnGR. For example, the status information for global mammalian and avian livestock breeds in the DAD-IS has increased from 43% and 39% respectively in 2009, to 57% and 48% in 2012 respectively (FAO, 2013). The effective monitoring of breed conservation status of livestock requires at least one census per generation of that target breed (FAO, 2007; Groeneveld *et al.*, 2010). Hence, a specific breed identification tool for each livestock breed will expedite such an exercise. However, pivotal to the success of these conservation efforts is the reliability of genetic identification of individual members within a target breed. The advancement in molecular technology in the last two decades has significantly increased our understanding the population genetics of domestic animals. It is apparent that the molecular characterisation of all domestic livestock breeds particularly in developing countries is a pre-requisite for their sustainable utilisation and conservation. This is because characterisation at the molecular level provides precise information for the determination of the actual population characteristics such as genetic variation and the effective population size (Luikart, England, Tallmon *et al.*, 2003). Currently, many domestic livestock breeds, particularly those in the developing countries, have yet to be characterised due to myriad issues including the lack of financial and technological capacity, as already described previously in this review. A recent report indicated that the risk status of 36% of all populations of local livestock breeds still remains unknown (FAO, 2013).

The main molecular technique used for most livestock genetic characterisation was microsatellite markers, for example they were was used in; Spanish native cattle breeds (Martín-Burriel, García-Muro & Zaragoza, 1999), Aberdeen Angus cattle breeds (Vasconcellos, Tambasco-Talhari, Pereira *et al.*, 2003), Austrian sheep breeds (Baumung, Cubric-Curik, Schwend *et al.*, 2006) and indigenous goats in sub-Saharan Africa (Chenyambuga, Hanotte, Hirbo *et al.*, 2004). Although highly informative, the current panels of microsatellites used for analyses are not capable of elucidating all the information required regarding breed variation in livestock (Toro *et al.*, 2009). Recently, it is

becoming more evident that SNP analysis is more suited for the high throughput genotyping that is required to elucidate greater molecular insights such as historical signatures of selection (Qanbari, Pausch, Jansen *et al.*, 2014), phenotypic variations within livestock breeds (Groenen, Megens, Zare *et al.*, 2011) as well as linkage disequilibrium over short physical distances (Kijas, Porto-Neto, Dominik *et al.*, 2014) than any of the other molecular markers currently available. The availability and accessibility of comprehensive databases of genomic data for various uses has also facilitated population genetic studies globally for example; the National Centre for Biotechnology Information(NCBI) (Sayers, Barrett, Benson *et al.*, 2011), Livestock Animal Quantitative Trait Loci (QTL) database (Hu, Park, Wu *et al.*, 2013), and University of California Santa Cruz (UCSC) genome browser (Dreszer, Karolchik, Zweig *et al.*, 2012). The challenge now is to use these enhanced insights and understanding of molecular methods to develop breed specific identification tools that are easily applicable to populations of livestock in different ecosystems. Such a breed-specific tool can be developed through identification and characterisation of unique phylogenomic SNPs in next generation sequenced pooled-genomic DNA from a minimum of 25 unrelated pure bred individuals. Information from any discovered phylogenomic SNPs can then be used to develop multiplexed SNP assays for the easy and precise identification of pure bred members from mixed populations of breeds in various ecosystems. These tools will not only facilitate the timely diagnosis of the conservation status of livestock breeds, but will also permit the regular monitoring of endangered breed populations, particularly in developing countries where the lack of technical and financial capacity is reported a major impediment.

## VII. CONCLUSIONS

(1) The importance of maintaining global domestic animal genetic diversity is important to human wellbeing.

(2) Breed specific molecular identification tools are urgently needed so that reliable and expeditious identification of individual members of any given breed can be achieved, and this is a pre-requisite for sustainable utilization and conservation of any breed.

(3) A growing number of studies have established that whole genome sequencing of pools of individuals within a group or breed provides great deal of information on genetic variation across the whole genome even when performed at relatively low coverage, but also at a considerable lower cost (Clarke *et al.*, 2014; Gautier, Foucaud, Gharbi *et al.*, 2013; Kim, Li, Guo *et al.*, 2010; Kofler *et al.*, 2015). This principle will be a cost effective technique for the identification of breed specific phylogenomic SNPs within a target breed for the purpose of developing a breed specific molecular identification tools.

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